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Evaluation of RSDL, M291 SDK, 0.5% Bleach, 1% Soapy Water and SERPACWA;

Part 2: Challenge with Soman

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14. ABSTRACT
Current doctrine describes the use of Skin Exposure Reduction Paste Against Chemical Warfare Agents (SERPACWA) as a barrier skin cream and the M291 Skin Decontamination Kit (SDK), 0.5% hypochlorite solution (household bleach diluted 1 to 10) and1% soapy water solution to decontaminate intact skin exposed to chemical warfare agents. Reactive Skin Decontamination Lotion (RSDL) is a new product approved by the FDA and selected in March 2007 by the Joint Program Executive Office for Chemical and Biological Defense to eventually replace the M291 SDK. This report, the second in a series, directly compares the efficacy of SERPACWA and the four listed decontamination products in the haired guinea pig model following exposure to soman (GD).

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EXECUTIVE SUMMARY

There is a need for Joint forces to effectively operate across the continuum of global contingency operations. The requirement exists for a pre-exposure barrier skin cream to increase the efficacy of the protective suit and for the ability to decontaminate the skin, individual equipment, and casualties, including those with wounds that have been exposed to chemical, biological, radiological, and nuclear (CBRN) warfare agents. Current doctrine describes the use of Skin Exposure Reduction Paste Against Chemical Warfare Agents (SERPACWA) as a barrier skin cream and the M291 Skin Decontamination Kit (SDK), 0.5% hypochlorite solution (household bleach diluted 1 to 10) and1% soapy water solution to decontaminate intact skin exposed to chemical warfare agents. Reactive Skin Decontamination Lotion (RSDL) is a new product approved by the FDA and selected in March 2007 by the Joint Program Executive Office for Chemical and Biological Defense to eventually replace the M291 SDK. This report, the second in a series, directly compares the efficacy of SERPACWA and the four listed decontamination products in the haired guinea pig model following exposure to soman (GD).

In all experiments, guinea pigs were close-clipped and given anesthesia. SERPACWA was applied as a thin coating (0. 1 mm thick), allowed to dry for 15 minutes and challenged with GD. After a 2-hour challenge any remaining GD was blotted off the animal, but no additional decontamination was done. In decontamination experiments, the animals were challenged with GD and decontaminated after a 2-minute delay for the standard procedure or at longer times for the delayed decontamination experiments. Positive control animals were challenged with GD in the same manner as the treated animals except that they received no treatment. All animals were observed during the first 4 hours and again at 24 hours postexposure for signs of toxicity and death. The protective ratio (PR, defined as LD_{50} of the treatment group divided by the LD_{50} of the untreated positive control animals) was calculated from the derived median lethal dose-response curves established for each treatment group and non-treated control animals. Significance in this report is defined as $p < 0.05$ unless otherwise stated. Neat GD was used to challenge all animals in these studies.

The results showed that SERPACWA provided significant, but modest, protection against GD with a PR of 2.1. In the standard 2-minute GD decontamination experiments, the calculated PRs for RSDL, 0.5% bleach, 1% soapy water, and M291 SDK were 14, 2.7, 2.2, and 2.7, respectively. RSDL was by far the most effective decontamination product tested and significantly better than any of the other products. Bleach, soapy water, and the M291 SDK provided equivalent and modest protection. Since only RSDL provided at least good protection (PR > 5) it was the only decontamination product evaluated for delayed decontamination. In the GD delayed decontamination experiments, the calculated LT_{50} value (the delayed decontamination time that 50% of the animals die in the test population following a 5 LD_{50} challenge) for RSDL was only 4.0 minutes. Unlike a VX challenge, effective decontamination following a GD challenge must be accomplished within the first few minutes of exposure.

Battelle Memorial Institute conducted a few similar, but not identical, efficacy evaluation experiments in a rabbit model. In a decontamination experiment both RSDL and the M291 SDK were evaluated. The PR values observed for RSDL (16) and the M291 SDK (4.7) in the Battelle study using rabbits were not statistically different ($p<0.05$) from the PRs (14 and 2.7) observed in our guinea pig model. The common-slopes probit model used to estimate the slopes for RSDL and the M291 SDK (5.23) in the Battelle study using rabbits, however, produced statistically different ($p<0.05$) slopes than the separate-slopes estimates for RSDL (11) and the M291 SDK (14) observed in our guinea pig model. In another rabbit study the Battelle group evaluated the efficacy of SERPACWA. In this study, however, there were very significant differences in the experimental procedure. SERPACWA was applied at a thickness of 0.15 mm vs. 0.10 mm, and the exposure site was decontaminated with 0.5% bleach at the end of the 2-hr exposure period. Most significantly, the applied GD was occluded with a polytetrafluoroethylene disk. The Battelle study determined that SERPACWA did not provide significant protection (PR = 1.1, $p>0.05$) when challenged with GD in the clipped guinea pig model. The PR was only slightly less than the marginal protection observed in our study (PR = 2.0). One would expect increased efficacy using a thicker application of barrier cream and decontamination with bleach. The observed results, however, demonstrate the importance of occluding the agent after challenge, which prevented agent evaporation, spread the GD over a larger surface area, and increased agent penetration through the barrier cream.

INTRODUCTION

This report, the second in a series, directly compares the efficacy of the four listed decontamination products and SERPACWA in the haired guinea pig model following exposure to soman (GD,1,2,2-trimethylpropyl methylphosphonofluoridate). Part 1 of the series (Braue et al., 2009) provided a detailed introduction to the decontamination products, SERPACWA, and the nerve agents, as well as to the threat nerve agents represent for warfighters and the civilian population.

OBJECTIVE

The first objective of this study was to determine the efficacy of four decontamination products in guinea pigs challenged with GD: the M291 SDK, 0.5% bleach, 1% soapy water, and RSDL. The second objective was to determine how the efficacy was affected by delaying application of these decontamination products following dermal GD challenge. The third objective was to determine the efficacy of the pretreatment barrier skin cream, SERPACWA. The fourth objective was to compare results in the guinea pig with results in the rabbit model.

MATERIALS AND METHODS

The materials and methods were completely described in Part 1 of this series (Braue et al., 2009). Any differences are outlined in this section.

Soman (GD, 1,2,2-trimethylpropyl methylphosphonofluoridate) was obtained from the U.S. Army Edgewood Chemical Biological Center (ECBC), Aberdeen Proving Ground, MD. The lot number was GD-U-2323-CTF-N and had a purity of 98.8% as determined by NMR spectroscopy.

In all experiments, GD was applied neat (undiluted with solvent). The maximum volume of GD that could be applied to SERPACWA protected skin without the agent running off the site was about 70 µl. If GD was observed to run off the SERPACWA protected site during the exposure period, the animal was excluded from the study results.

RESULTS

Tables 1-3 provide a summary of the SAS probit analysis for all of the experiments. It includes, for each treatment, the number of animals, the LD_{10}, LD_{50} and LD_{90} (LT_{10}, LT_{50} and LT_{90} for delayed decontamination experiments), the lower and upper 95% CI, the dose-response curve slope, and the PR. Within a given experimental group, the SAS analysis using the Delta method determined which PRs were significantly different at both the 95 and 99.5% levels. PRs with same letter were not statistically different.

The LD_{50} values for control, 0.5% bleach, M291 SDK, RSDL, and 1% soapy water were 11, 29, 30, 154, and 24 mg/kg, respectively, and are presented graphically in Figure 1. The error bars represent the 95% CI. The number of animals used per treatment group was between 20 and 30. All animals were challenged with neat GD. Figure 2 is a graph of PR values calculated from the data in Figure 1. PR values with the same letter were not statistically different at the 0.05 decision level. The PR values for 0.5% bleach, M291 SDK, RSDL, and 1% soapy water were 2.6, 2.7, 14, and 2.2, respectively.

The LD_{50} values for control and SERPACWA were 17 and 35 mg/kg, respectively, and are presented graphically in Figure 3. The error bars represent the 95% CI. The numbers of animals used for positive controls and SERPACWA were 30 and 34, respectively. All animals were challenged with neat GD. Figure 4 is a graph of protective ratio (PR) values calculated from the data in Figure 3. PR values with the same letter were not statistically different at the 0.05 decision level. The PR value for SERPACWA challenged with neat GD was 2.1.

Figure 5 is a graph of percent lethality when RSDL decontamination is delayed following challenge by 55.5 mg/kg (5 LD_{50}s) of neat GD. The LT_{50} (50% lethality time) was 4.0 minutes with a 95% CI of 3.9 to 4.1 minutes. The probit slope was 93 using a total of 48 animals.

The raw data for all experiments are presented in Appendix A. Tables A1- A5 provide the raw data for the standard decontamination experiments (decontamination 2 min postexposure). These tables provide the 24-hour survival data for positive control animals and animals decontaminated with 0.5% bleach, M291 SDK, RSDL, and 1% soapy water. Tables A6 and A7 provide the survival raw data for the SERPACWA experiments. These tables provide the 24-hour survival data for positive control animals and animals pretreated with SERPACWA. Table A8 provides the survival raw data for the delayed decontamination experiments. In these experiments, the decontamination process was delayed from 2 to 180 minutes postexposure. All of these animals were challenged with 55.0 mg/kg neat GD, which represents a 5 LD_{50} dosage.

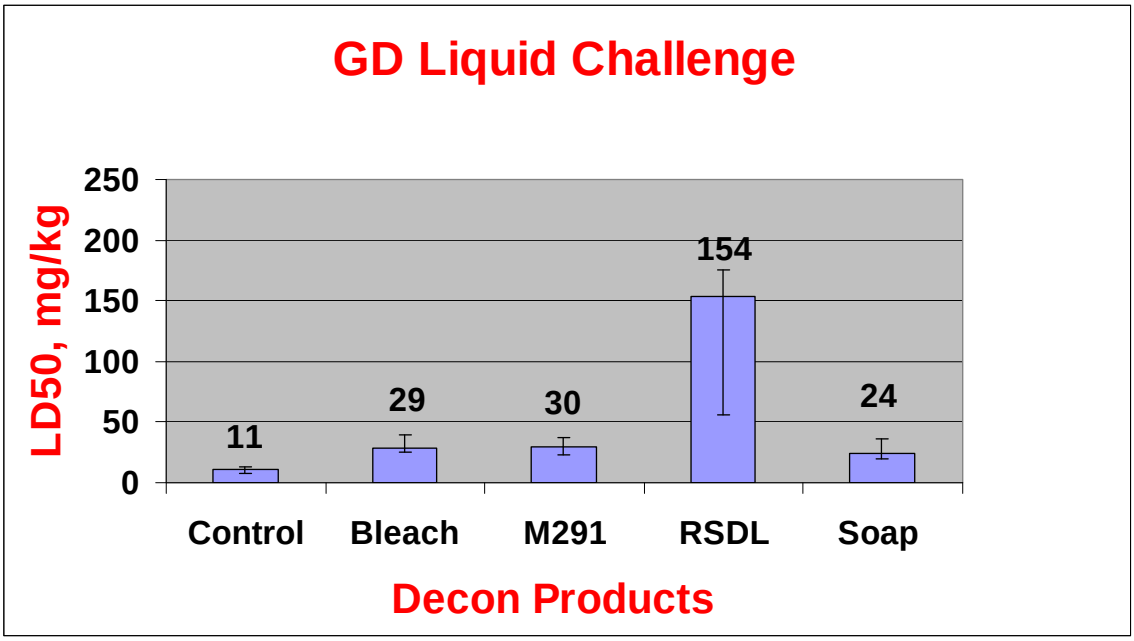

Figure 1. Graph of LD_{50} values for decontamination products in guinea pig model. Error bars = 95% CI. The number of animals used per treatment group was 20 - 30. All animals were challenged with neat GD.

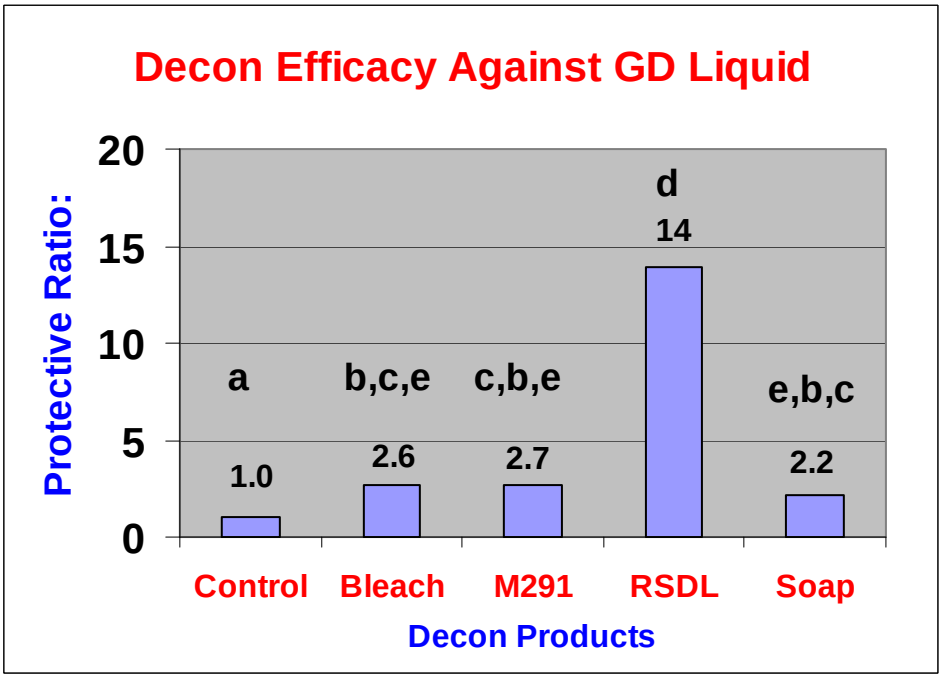

Figure 2. Graph of protective ratio (PR) values for decontamination products in guinea pig model. The number of animals used per treatment group was 20 - 30. All animals were challenged with neat GD. PRs with same letter were not statistically different at the 0.05 decision level.

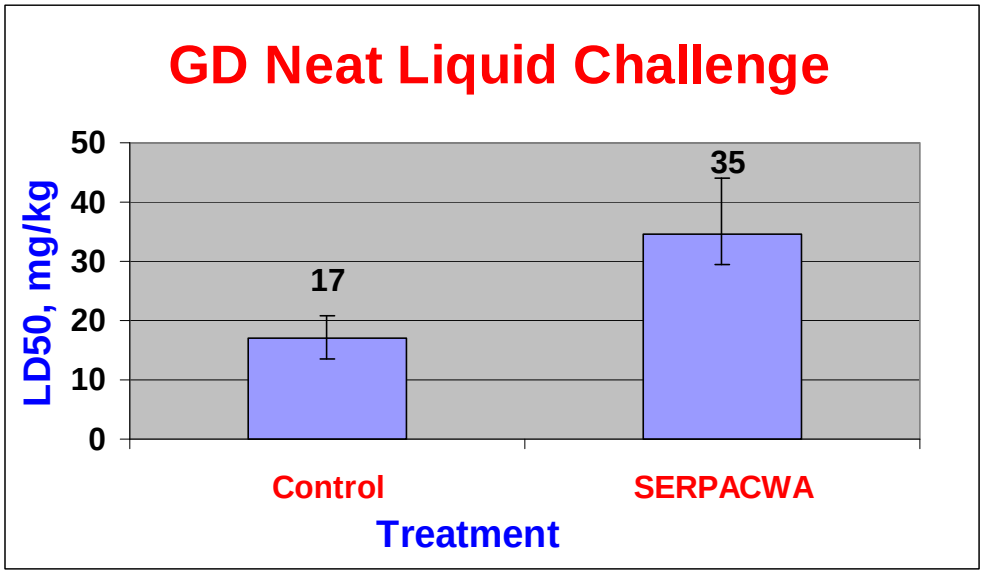

Figure 3. Graph of LD_{50} values for positive controls and SERPACWA in the guinea pig model. Error bars = 95% CI. Thirty animals were used for positive controls and 34 animals for SERPACWA. All animals were challenged with neat GD.

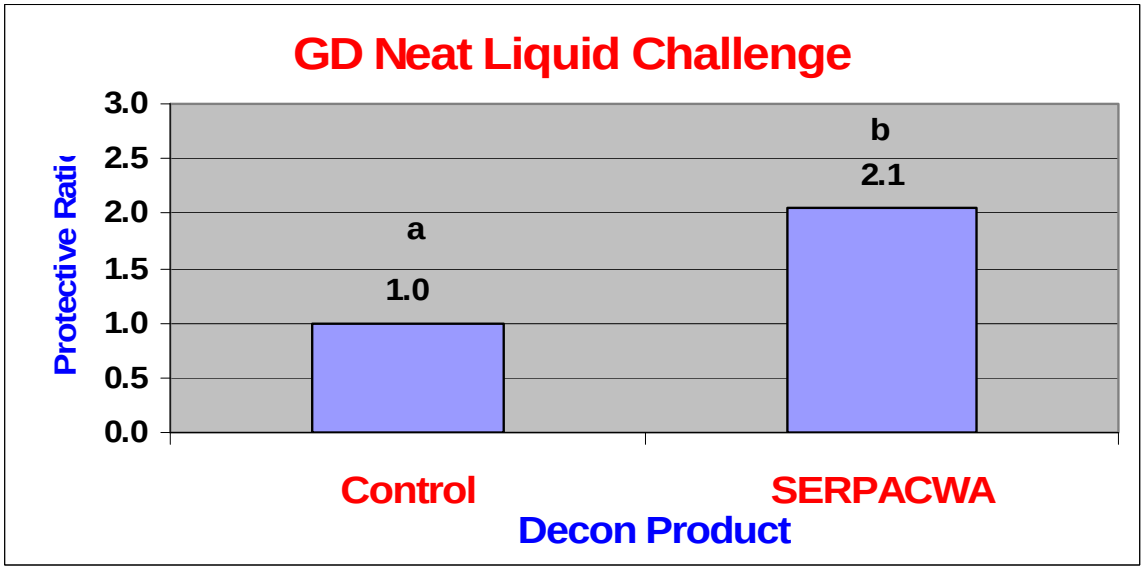

Figure 4. Graph of PR values for positive controls and SERPACWA in the guinea pig model. Thirty animals were used for positive controls and 34 animals for SERPACWA. All animals were challenged with neat GD. PRs with same letter were not statistically different at the 0.05 decision level.

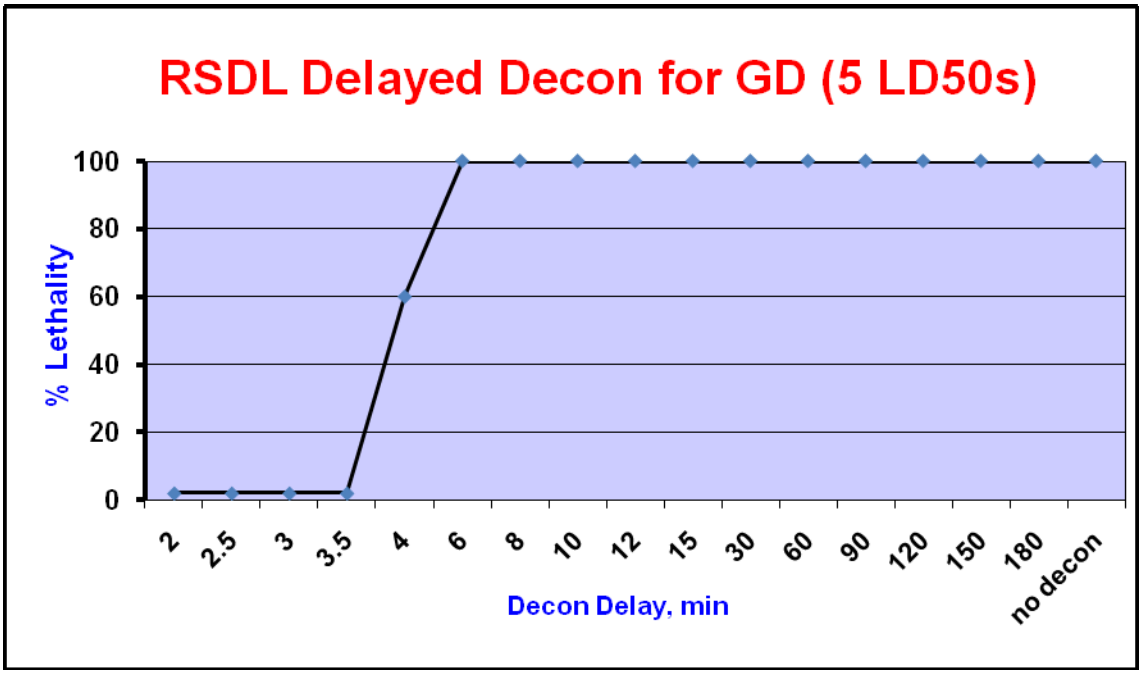

Figure 5. Graph of Percent lethality when RSDL decontamination was delayed following challenge by 55.5 mg/kg (5 LD_{50}s) of GD. The LT_{50} (50% lethality time) was 4.0 minutes with a 95% CI. of 3.9 to 4.1 minutes. The probit slope was 93 using a total of 48 animals.

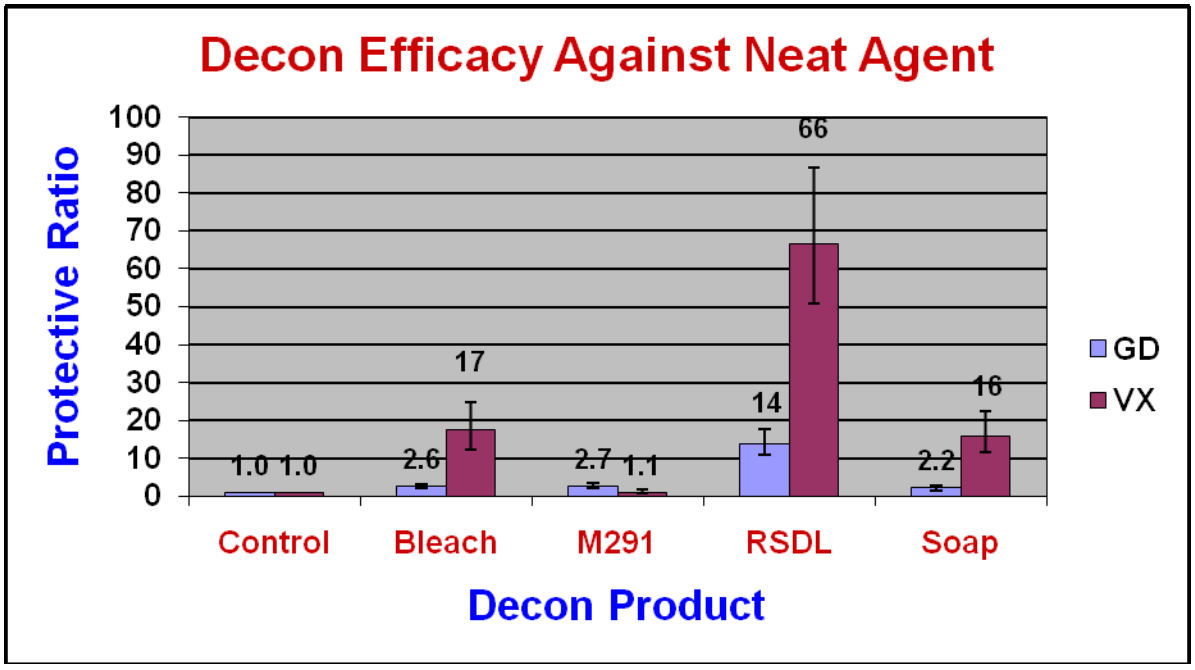

Figure 6. Graph comparing protective ratio (PR) values for decontamination products challenged with GD and VX in guinea pig model. Error bars = 95% CI.

AGENT	No. of G.P.	Treat-ment	LD_{50} mg/kg	LCL	UCL	Slope	PR	PR 95% CI	95% Sig	99.5 % Sig	LD_{10}	LCL	UCL	LD_{90}	LCL	UCL
GD Neat	29	Control	11.0	7.71	13.5	7.62	1.00		a	a	7.47	2.29	9.50	16.2	13.3	37.6
GD Neat	23	Bleach	29.0	24.7	40.0	21.7	2.63	2.14 – 3.25	b,c,a	b,c,a	25.3	6.14	27.7	33.2	30.0	191
GD Neat	20	M291	30.0	22.8	37.8	13.6	2.73	2.16 - 3.44	c,b,e	c,b,e	24.2	6.75	28.0	37.2	32.2	122
GD Neat	22	RSDL	154	55.4	11.1	11.1	14.0	11.0 - 17.7	d	d	118	1.68	142	200	175	2285
GD Neat	30	Soap	24.0	19.7	6.58	6.58	2.18	1.64 - 2.89	e,b,c	e,b,c	15.3	7.28	18.8	37.5	28.5	128

Table 1. Data summary of efficacy experiments for decontamination products with animals challenged with GD. Includes decontamination experiments from 29 March 2005 to 26 April 2005.

AGENT	No. of G.P.	Treatment	LT_{50} min	LCL	UCL	Slope	LT_{10}	LCL	UCL	LT_{90}	LCL	UCL
GD Neat	48	RSDL delayed	3.98	3.87	4.09	92.7	3.85	3.74	3.96	4.10	3.99	4.21

Table 2. Data summary of efficacy experiments for delayed decontamination with animals challenged with GD. Includes delayed decontamination experiments from 2 August 2007 to 15 November 2007.

AGENT	No. of GP	Treatment	LD_{50}	LCL	UCL	Slope	PR	PR 95% CI	95% Sig	99.5 % Sig	LD_{10}	LCL	UCL	LD_{90}	LCL	UCL
GD Neat	30	Control	16.9	13.6	20.9	8.3	1		a	a	11.8	6.44	14.4	24.1	19.8	44.3
GD Neat	34	SERPACWA	34.6	29.4	44.0	8.4	2.05	1.63 – 2.58	b	b	24.3	13.8	28.9	49.3	40.4	104

Table 3. Data summary of efficacy experiments for SERPACWA with animals challenged with neat GD. Includes experiments from 24 August 2005 to 15 September 2005.

Notes for Tables 1-3:

- LD_{10}, LD_{50}, and LD_{90} = the dosage (mg/kg body weight) required to kill 10, 50, and 90% respectively of the test population.
- LT_{10}, LT_{50}, and LT_{90} = the delayed decontamination time at which 10, 50, and 90% of the animals in the test population die following a 55.5 mg/kg (5 LD_{50}) challenge.
- LCL = Lower confidence limit at $p < 0.05$ (Fieller's method).
- UCL = Upper confidence limit at $p < 0.05$ (Fieller's method).
- PR = Protective ratio (LD_{50} of treatment/LD_{50} of control).
- PR 95% CI = Protective ratio 95% confidence interval (Delta method).
- 95% Sig = Protective ratios with same letter were not statistically different at the 0.05 decision level (Delta method).
- 99.5% Sig = Protective ratios with same letter were not statistically different at the 0.005 decision level (Delta method).

Slope = The probit analysis slope.

DISCUSSION

This report is the second in a series to provide a comprehensive comparison of the efficacy of these decontamination products and SERPACWA against most of the traditional chemical warfare agents.

The real world threat scenario is for exposure to neat agent not agent in solution. In our initial VX experiments (Braue et al., 2009) the toxicity of VX was so great that some solution challenges had to be conducted. The toxicity of GD in guinea pigs, however, allowed all animals to be dosed with neat GD.

In the initial series of experiments with GD, the calculated PRs for the standard 2-minute decontamination experiments with RSDL, 0.5% bleach, 1% soapy water, and M291 SDK (solution) were 14, 2.6, 2.2, and 2.7, respectively (Table 1). RSDL was by far the most effective decontamination product tested and significantly better than any of the other products. Bleach, soapy water, and the M291 SDK provided only modest protection and were not statistically different from each other.

The LD_{50} value is traditionally used to compare the toxicity of chemicals; however, the probit slope from the dose-response curve is also an important parameter to indicate how quickly the percent lethality changes with applied dose. If the probit slope is flat, the percent of lethality changes very slowly with changes in dose, and a significant percentage of deaths is observed at doses far removed from the median lethal dose. The PROBSEP program run in SAS to analyze this data set not only provided the LD_{50} values but also gave doses for the complete range of lethality percentiles including 1, 10, 16, 30, 50, 70, 84, 90, and 99. The doses for this entire range are recorded in the lab notebooks but are not provided in this report. We did provide (Table 1) the slope, LD_{10} LD_{50}, and LD_{90} values along with the 95% CI values so that the reader can fully understand the toxicity of GD and the effectiveness of the products tested.

In these decontamination experiments, all of the slopes were relatively large with values of 7.6, 11, 22, 6.6, and 14 for positive controls, RSDL, 0.5% bleach, 1% soapy water, and M291 SDK, respectively. Although the LD_{50} values have been traditionally used as the measure of toxicity, knowing the LD_1 or LD_{10} value may be more relevant. Knowing the dose at which very few or no deaths are expected may be the most relevant information. The LD_{50} value is generally used for toxicity assessment because its location on the dose response curve is where the most information is collected and, thus, it has the smallest CI. If the LD_{10} values are used to calculate a modified PR the resulting PRmod values for RSDL, bleach, soapy water, and M291 SDK (neat) are 16, 3.3, 2.0, and 3.2, respectively. These values within statistical significance are similar to the PRs calculated (14, 2.6, 2.2, and 2.7) using the LD_{50} values, as reflected in their similar slopes (see Table 1).

Battelle Memorial Institute (Columbus, OH) conducted one evaluation of decontamination products challenged with GD, sponsored by the U.S. Army Medical

Research and Materiel Command. This evaluation was conducted using the same general methodology as the experiments described in this report except that the animal model was the rabbit (New Zealand White, male). This study, conducted in February 2004 (Babin et al., 2004, Battelle Task 017), involved a direct comparison of the efficacy of RSDL and M291 SDK vs. positive control animals. The results are summarized below.

Decon System	Total No. of Animals	Common-Slopes Probit Model		GD LD_{50} Dose (mg/kg)	Fieller's 95% Confidence Interval	Delta Method PR	
		Slope Estimate (Std.) Error)	P-value for Signif. of slope			Protective Ratio	95% CI
RSDL	16	5.23 (1.43)	0.0003	27.1	19.1 – 44.8	16	9.1 – 26.9
M291 SDK	14			8.0	4.8 – 12.4	4.7	2.0 – 5.8
None	18			1.7	1.1 – 2.8	1	NR

Table 4. Data summary of efficacy evaluation studies for RSDL and M291 SDK conducted at Battelle Memorial Institute in February 2004 (Babin et al., 2004, Battelle Task 17). Std. = standard deviation. NR = not reported.

The PRs observed for RSDL (16) and the M291 SDK (4.7) in the Battelle study using rabbits were not statistically different from the PRs (14 and 2.7) observed in our guinea pig model. The common-slopes probit model used to estimate the slopes for RSDL and the M291 SDK (5.23) in the Battelle study using rabbits, however, produced widely different slopes from the separate-slopes estimate for RSDL (11 with a 95% CI of 0 – 22) and the M291 SDK (14 with a 95% CI of 1.0-26). Although widely different slope values were observed in the two studies they were not statistically different because of the large CI values observed in the guinea pig studies.

SERPACWA was observed to provide modest, but significant ($p< 0.05$), protection against GD in our guinea pig model with a PR of 2.0. The observed LD_{50} values for positive control and SERPACWA animals were 17 and 35, respectively. The observed slopes for positive control and SERPACWA animals were equivalent and steep with values of 8.3 and 8.4, respectively.

Throughout the development of the SERPACWA final formulation, a 4-hour challenge was considered the standard agent exposure time to use for efficacy evaluations. At Battelle, male rabbits in the weight range of 2 to 4 kg were used for most of the *in vivo* studies, and this model allowed the animals to be kept under

anesthesia for 4 hours. Male guinea pigs with their lower body weight of 250 – 400 g, however, could not be kept under anesthesia for 4 hours without a high mortality rate. Preliminary experiments determined that 2 hours was about as long as these animals could be safely kept under anesthesia, so this time was used for the agent exposure in guinea pigs (Snider et al., 2005, Battelle Task 003, Module 9).

Battelle conducted a male guinea pig study to evaluate SERPACWA (Snider et al., 2005, Battelle Task 003, Module 9) from 2 December 2002 to 18 December 2003. The testing procedure was different from the method described in this report. SERPACWA was spread at a thickness of 0.15 mm instead of 0.10 mm, after application of GD the sites were occluded with a 32-mm diameter polytetrafluoroethylene disk, and after the 2-hour exposure period the sites were wiped with a dry gauze to remove the agent and SERPACWA, followed by two skin decontaminations with 10% Ca $(OCl)_2$ solution and two more decontaminations with water. The results from the Battelle guinea pig study are summarized below.

Pretreatment	Total No. of Animals	Probit Dose-Response Slope		GD LD_{50} Dose (mg/kg)	Fieller's 95% Confidence Interval	Delta Method PR	
		Slope Estimate	95% Confidence Interval			Protective Ratio	P-value for Signif. of PR
None	30	1.5	0.3 – 2.7	30	6.4 – 80	1	NR
SERPACWA	17	2.7	0.1 – 5.4	32	5.7 – 1432	1.1	NR

Table 5. Data summary of efficacy evaluation studies for SERPACWA conducted at Battelle Memorial Institute from December 2002 to December 2003 (Snider et al., 2005, Battelle Task 003, Module 9). NR = not reported.

The Battelle study determined that SERPACWA did not provide significant protection (PR = 1.1, $p>0.05$) when challenged with GD in the guinea pig model. The PR was only slightly less than the marginal protection observed in our study (PR = 2.0). It is surprising, however, that the Battelle study did not demonstrate better efficacy, especially considering the differences in the experimental procedure. The thicker application of SERPACWA (0.15 vs. 0.10 mm) and the decontamination process at the end of the exposure should have resulted in higher efficacy not lower. When SERPACWA was challenged with VX (Snider et al., 2005, Battelle Task 003, Module 6) using a procedure that removed SERPACWA and decontaminated the exposure site, Battelle observed very high efficacy (PR = 66). A possible explanation for this observation is the fact that the exposure site was occluded after application of GD but not occluded in the VX study. A high percentage of an applied dose of GD has been suggested to evaporate from the skin before skin penetration, but a literature search did not produce any experimental evidence to support this claim. Early work on the

absorption and distribution of sulfur mustard (Renshaw, 1946; Cullumbine, 1947) indicated that for an applied dose of sulfur mustard, about 80% of the agent evaporates and only 20% is absorbed into the skin. Both sulfur mustard and GD are lipophilic agents and are readily absorbed into the skin. They also have similar volatility with boiling points of 218 and 198 degrees C for sulfur mustard and GD, respectively. Perhaps with these similarities a reasonable assumption is that a majority of the applied GD dose would evaporate before it had a chance to penetrate through the SERPACWA into the skin. Occluding the GD application site with a polytetrafluoroethylene disk would have two effects. First it would spread the agent out over a greater surface area, and second it would limit evaporation. Both of these actions would tend to increase the severity of the GD challenge. These factors may provide an explanation of why the Battelle study observed no protection by SERPACWA when the GD application site was occluded.

In a real-life scenario, warfighters or civilians may not realize that they have been contaminated with a toxic agent. Thus, they may not start the decontamination process until well after the recommended time of 1 or 2 minutes postexposure. The conventional wisdom for many years was that decontamination would only be effective if performed in the first few minutes after exposure. When this study started in fiscal year (FY) 2005, there were literally no comprehensive evaluations available on the effectiveness of decontamination products beyond the standard 2-minute delay time. A limited study (Hamilton et al., 2004) using only 3 animals per treatment group evaluated VX decontamination with RSDL in swine (Yorkshire-Landrace cross, 20 kg). In this study, RSDL was found to be significantly effective 15 minutes postexposure for neat VX challenge to the ear but not significantly effective 30 or 60 minutes postexposure for neat VX challenge to the epigastrium (belly). Recognizing the need for a comprehensive study, the scope our current study was expanded to include delayed decontamination studies for all agents.

A fixed challenge dose of 55.0 mg/kg (5 LD_{50}) GD was used for all delayed decontamination studies. This dose was selected because historically a 5 LD_{50} dose was the suggested minimum target for therapeutics selected for fielding. The lethality delay time-response curves were generated using the sequential stage-wise method similar to the LD_{50} dose-response curves using the delay time in place of the mg/kg dose. The standard probit analysis program was used to find the lethality percentiles associated with a given decontamination delay time. The LT_{10}, LT_{50}, and LT_{90} values were defined as the delayed decontamination times at which 10, 50, and 90% of the animals in the test population died following a 55.0 mg/kg (5 LD_{50}) challenge. A PR of 5, which is directly related to protection from a 5 LD_{50} challenge, was the decision criteria for choosing the decontamination products for the delayed decontamination experiments. Any decontamination products with a PR > 5 would be evaluated for delayed decontamination. For the GD experiments only RSDL met this requirement.

RSDL was evaluated for delayed decontamination following GD challenge. The experiments were conducted after we had completed the delayed decontamination experiments with VX (Braue et al., 2009). Against VX, we observed an LT_{50} of 31

minutes, indicating a window of opportunity for delayed decontamination. With GD, however, the LT_{10}, LT_{50}, and LT_{90} values were observed to be 3.9, 4.0, and 4.1, respectively. These values reflect the very steep slope of 93. Using the most effective decontamination product, RSDL, decontamination had to be accomplished very soon after exposure. There was virtually no window for delayed decontamination after exposure to GD.

A goal of this project was for it to be a bridging study between the early results observed in a rabbit model and the results observed in the guinea pig model. Using a GD challenge, we could identify only one study in a rabbit model that was similar to our experiments using guinea pigs. The study, conducted by Battelle, is summarized above (Snider et al., 2005, Task 003, Module 9). The results demonstrated excellent correlation between the PR values for both RSDL (16 vs. 14) and the M291 SDK (4.7 vs. 2.7). There was also good correlation between the observed slopes for the control animals in the rabbit experiments (5.2) and the guinea pig experiments (7.6). The correlation between the slopes for RSDL and the M291 SDK, however, were not as good. In the rabbit experiments, which used a common-slopes probit model, the observed slope was the same as with the control animals, 5.2. In the guinea pig experiments, which used a separate-slopes probit model, the observed slopes for RSDL and M291 SDK were 11.1 (95% CI = 1.8-13.4) and 13.6 (95% CI = 1.0-26.3). The slope values were only slightly different, and each slope value was within the 95% CI of the other and therefore not statistically significantly different. For challenge with GD, the data generated in the rabbit and guinea pig models suggest that either model would give similar relative efficacy results when evaluating decontamination products.

GD remains an agent of real concern. Even though the percutaneous toxicity of GD is about 50-90 times less than that of VX (LD_{50} of GD = 11 mg/kg; LD_{50} of VX = 0.12 – 0.22 mg/kg, Braue et al., 2009), decontaminating GD is very difficult. The standard decontamination products, 0.5% bleach and 1% soapy water, only have PR values of 2.6 and 2.2, respectively, and these are significantly lower than those for VX (Figure 6). While RSDL provides good protection with a PR of 14, there is only a very brief window of about 2-3 minutes where this protection is observed. Postexposure, GD is also very difficult to treat with the standard therapies of atropine, 2-PAM, (2-[(hydroxyimino)methyl]-1-methylpyridinium dichloride), and benzodiazepines, primarily because of rapid aging of the organophosphorus-acetylcholinesterase conjugate and the inability of an oxime to reactivate free acetylcholinesterase (Maxwell et al., 2008).

CONCLUSIONS

- RSDL provided superior protection against GD compared to the other products tested.
- 0.5% bleach, 1% soapy water, and the M291 SDK were less effective than RSDL, but still provided modest (2< PR < 5) protection against GD.
- RSDL, the best product tested, did not provide significant protection against GD when decontamination was delayed for more than 3 minutes.
- SERPACWA provided significant, but modest, protection against GD.
- There was good correlation between using the rabbit model and the guinea pig model for decontamination efficacy evaluations.
- GD is an agent of real concern because it is very difficult to decontaminate and the effects of exposure are difficult to treat.

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APPENDIX A

EXPERIMENTAL RAW DATA

Agent	Date	Treatment	Dose mg/kg	Log Dose	Number Animals	Number Dead	Time to Death (Hours)
GD Neat	29-Mar-05	Control	2	0.301	1	0	>24
GD Neat	31-Mar-05	Control	3	0.477	1	0	>24
GD Neat	4-Apr-05	Control	3	0.477	1	0	>24
GD Neat	31-Mar-05	Control	4.7	0.672	1	0	>24
GD Neat	4-Apr-05	Control	4.7	0.672	1	0	>24
GD Neat	12-Apr-05	Control	6.3	0.799	1	0	>24
GD Neat	12-Apr-05	Control	7.9	0.898	1	0	>24
GD Neat	26-Apr-05	Control	7.9	0.898	2	0	>24
GD Neat	12-Apr-05	Control	10	1	1	1	4
GD Neat	14-Apr-05	Control	10	1	1	0	>24
GD Neat	26-Apr-05	Control	10	1	2	1	1, >24
GD Neat	19-Apr-05	Control	11.9	1.076	1	1	2
GD Neat	21-Apr-05	Control	11.9	1.076	3	3	3, O/N, O/N
GD Neat	31-Mar-05	Control	14	1.146	1	1	2
GD Neat	4-Apr-05	Control	14	1.146	1	0	>24
GD Neat	14-Apr-05	Control	14	1.146	2	0	>24
GD Neat	19-Apr-05	Control	14.9	1.173	2	2	2, 2
GD Neat	6-Apr-05	Control	16	1.204	2	2	1, 1
GD Neat	29-Mar-05	Control	20	1.301	1	1	O/N
GD Neat	6-Apr-05	Control	20	1.301	1	1	1
GD Neat	26-Apr-05	Control	20	1.301	2	2	1, 1

Table A1. Raw data for positive control animals challenged with GD in the decontamination product experiments. O/N = overnight (6-20 hrs).

Agent	Date	Treatment	Dose mg/kg	Log Dose	Number Animals	Number Dead	Time to Death (Hours)
GD Neat	31-Mar-05	Bleach	11	1.041	1	0	>24
GD Neat	04-Apr.05	Bleach	11	1.041	1	0	>24
GD Neat	4-Apr-05	Bleach	14.8	1.17	1	0	>24
GD Neat	12-Apr-05	Bleach	15.8	1.199	1	0	>24
GD Neat	12-Apr-05	Bleach	20	1.301	2	0	>24
GD Neat	4-Apr-05	Bleach	21.7	1.336	1	0	>24
GD Neat	14-Apr-05	Bleach	21.7	1.336	1	0	>24
GD Neat	6-Apr-05	Bleach	25	1.398	2	1	O/N, >24
GD Neat	14-Apr-05	Bleach	25	1.398	2	0	>24
GD Neat	19-Apr-05	Bleach	28.2	1.45	3	0	>24
GD Neat	21-Apr-05	Bleach	28.2	1.45	1	0	>24
GD Neat	31-Mar-05	Bleach	32	1.505	1	1	2
GD Neat	6-Apr-05	Bleach	32	1.505	1	1	O/N
GD Neat	21-Apr-05	Bleach	32	1.505	2	2	2, 2
GD Neat	31-Mar-05	Bleach	56	1.748	1	1	1
GD Neat	29-Mar-05	Bleach	100	2	1	1	1
GD Neat	29-Mar-05	Bleach	300	2.477	1	1	1

Table A2. Raw data for 0.5% bleach animals challenged with GD. O/N = overnight (6-20 hrs).

Agent	Date	Treatment	Dose mg/kg	Log Dose	Number Animals	Number Dead	Time to Death (Hours)
GD Neat	29-Mar-05	M291	12.5	1.097	1	0	>24
GD Neat	12-Apr-05	M291	20	1.301	1	0	>24
GD Neat	14-Apr-05	M291	20	1.301	1	0	>24
GD Neat	12-Apr-05	M291	25	1.398	2	1	O/N, >24
GD Neat	14-Apr-05	M291	25	1.398	2	0	>24
GD Neat	19-Apr-05	M291	28.2	1.45	1	0	>24
GD Neat	29-Mar-05	M291	32	1.505	1	0	>24
GD Neat	6-Apr-05	M291	32	1.505	1	1	3
GD Neat	19-Apr-05	M291	32	1.505	2	1	2, >24
GD Neat	6-Apr-05	M291	35.5	1.55	2	2	1, 2
GD Neat	31-Mar-05	M291	40	1.602	1	1	2
GD Neat	4-Apr-05	M291	40	1.602	1	1	O/N
GD Neat	31-Mar-05	M291	50	1.699	1	1	1
GD Neat	4-Apr-05	M291	50	1.699	1	1	1
GD Neat	31-Mar-05	M291	63	1.799	1	1	1
GD Neat	4-Apr-05	M291	63	1.799	1	1	1

Table A3. Raw data for M291 SDK animals challenged with GD. O/N = overnight (6-20 hrs).

Agent	Date	Treatment	Dose mg/kg	Log Dose	Number Animals	Number Dead	Time to Death (Hours)
GD Neat	31-Mar-05	RSDL	63	1.799	1	0	>24
GD Neat	31-Mar-05	RSDL	100	2	1	0	>24
GD Neat	21-Apr-05	RSDL	125	2.097	2	0	>24
GD Neat	31-Mar-05	RSDL	158	2.199	1	0	>24
GD Neat	4-Apr-05	RSDL	158	2.199	1	0	>24
GD Neat	14-Apr-05	RSDL	158	2.199	2	1	4, >24
GD Neat	21-Apr-05	RSDL	158	2.199	3	3	3, 4, O/N
GD Neat	19-Apr-05	RSDL	168	2.225	3	3	3, O/N, O/N
GD Neat	4-Apr-05	RSDL	178	2.25	1	1	2
GD Neat	6-Apr-05	RSDL	178	2.25	1	1	3
GD Neat	12-Apr-05	RSDL	178	2.25	1	1	3
GD Neat	14-Apr-05	RSDL	178	2.25	1	0	>24
GD Neat	4-Apr-05	RSDL	200	2.301	1	0	>24
GD Neat	6-Apr-05	RSDL	200	2.301	1	1	2
GD Neat	12-Apr-05	RSDL	200	2.301	2	2	O/N, O/N
GD Neat	29-Mar-05	RSDL	250	2.398	1	1	O/N
GD Neat	6-Apr-05	RSDL	250	2.398	1	1	2
GD Neat	29-Mar-05	RSDL	630	2.799	1	1	1

Table A4. Raw data for RSDL animals challenged with GD. O/N = overnight (6-20 hrs).

Agent	Date	Treatment	Dose mg/kg	Log Dose	Number Animals	Number Dead	Time to Death (Hours)
GD Neat	26-Apr-05	Soap	6.3	0.799	1	0	>24
GD Neat	31-Mar-05	Soap	11	1.041	1	0	>24
GD Neat	4-Apr-05	Soap	11	1.041	1	0	>24
GD Neat	4-Apr-05	Soap	14.8	1.17	1	0	>24
GD Neat	12-Apr-05	Soap	14.8	1.17	1	0	>24
GD Neat	26-Apr-05	Soap	14.8	1.17	2	0	>24
GD Neat	14-Apr-05	Soap	15.8	1.199	2	1	O/N, >24
GD Neat	19-Apr-05	Soap	15.8	1.199	2	0	>24
GD Neat	6-Apr-05	Soap	17.9	1.253	2	1	O/N, >24
GD Neat	12-Apr-05	Soap	17.9	1.253	2	0	>24
GD Neat	19-Apr-05	Soap	19.7	1.294	1	0	>24
GD Neat	21-Apr-05	Soap	19.7	1.294	3	0	>24
GD Neat	4-Apr-05	Soap	21.7	1.336	1	1	3
GD Neat	6-Apr-05	Soap	21.7	1.336	1	1	O/N
GD Neat	14-Apr-05	Soap	25	1.398	1	1	O/N
GD Neat	21-Apr-05	Soap	25	1.398	1	0	>24
GD Neat	26-Apr-05	Soap	25	1.398	2	1	4, >24
GD Neat	31-Mar-05	Soap	32	1.505	1	1	1
GD Neat	26-Apr-05	Soap	32	1.505	2	1	2, >24
GD Neat	26-Apr-05	Soap	39.8	1.6	2	2	1, O/N
GD Neat	31-Mar-05	Soap	56	1.748	1	1	1
GD Neat	29-Mar-05	Soap	100	2	1	1	1
GD Neat	29-Mar-05	Soap	300	2.477	1	1	1

Table A5. Raw data for 1% soapy water animals challenged with GD. O/N = overnight (6-20 hrs).

Agent	Date	Treatment	Dose mg/kg	Log Dose	Number Animals	Number Dead	Time to Death (Hours)
GD Neat	24-Aug-05	Control	7.72	0.8876	2	0	>24
GD Neat	15-Sep-05	Control	7.72	0.8876	2	0	>24
GD Neat	15-Sep-05	Control	8.9	0.9494	2	0	>24
GD Neat	24-Aug-05	Control	11	1.0414	2	0	>24
GD Neat	1-Sep-05	Control	11	130414	2	0	>24
GD Neat	24-Aug-05	Control	13.5	1.1303	2	1	O/N, >24
GD Neat	30-Aug-05	Control	13.5	1.1303	2	0	>24
GD Neat	30-Aug-05	Control	153800	1.1987	2	2	2, 2
GD Neat	1-Sep-05	Control	15.8	1.1987	2	1	4, >24
GD Neat	30-Aug-05	Control	20	1.301	2	1	2, >24
GD Neat	1-Sep-05	Control	20	1.301	2	0	>24
GD Neat	15-Sep-05	Control	22.4	1.3502	2	2	2, 2
GD Neat	1-Sep-05	Control	25	1.3979	2	2	3, 28
GD Neat	15-Sep-05	Control	25	1.3979	2	2	2, 2
GD Neat	15-Sep-05	Control	30	1.4771	2	2	1, 2

Table A6. Raw data for positive control animals challenged with GD in the SERPACWA experiments. O/N = overnight (6-20 hrs).

Agent	Date	Treatment	Dose mg/kg	Log Dose	Number Animals	Number Dead	Time to Death (Hours)
GD Neat	24-Aug-05	SERPACWA	7.72	0.8876	2	0	>24
GD Neat	24-Aug-05	SERPACWA	11	1.0414	2	0	>24
GD Neat	24-Aug-05	SERPACWA	22	1.3424	2	0	>24
GD Neat	30-Aug-05	SERPACWA	22	1.3424	2	0	>24
GD Neat	30-Aug-05	SERPACWA	24.5	1.3892	2	1	1, >24
GD Neat	1-Sep-05	SERPACWA	24.5	1.38.92	2	0	>24
GD Neat	30-Aug-05	SERPACWA	27.5	1.4393	2	0	>24
GD Neat	1-Sep-05	SERPACWA	27.5	1.4393	2	1	O/N, >24
GD Neat	30-Aug-05	SERPACWA	30.9	1.49	2	1	3, >24
GD Neat	1-Sep-05	SERPACWA	30.9	1.49	2	1	29, >24
GD Neat	30-Aug-05	SERPACWA	34.7	1.5403	2	0	>24
GD Neat	1-Sep-05	SERPACWA	34.7	1.5403	2	0	>24
GD Neat	15-Sep-05	SERPACWA	38.9	1.5899	2	1	O/N, >24
GD Neat	24-Aug-05	SERPACWA	44	1.6435	2	2	2, 4
GD Neat	15-Sep-05	SERPACWA	44	1.6435	2	2	2, O/N
GD Neat	15-Sep-05	SERPACWA	53.7	1.73	2	2	4, O/N
GD Neat	24-Aug-05	SERPACWA	66	1.8195	2	2	2, O/N

Table A7. Raw data for SERPACWA animals challenged with GD. O/N = overnight (6-20 hrs).

Agent	Date	Treatment	Time Delay, min	Number Animals	Number Dead	Time to Death (Hours)
GD Neat	2-Aug-07	RSDL delayed	2	2	0	>24
GD Neat	8-Nov-07	RSDL delayed	2	2	0	>24
GD Neat	15-Nov-07	RSDL delayed	2	3	0	>24
GD Neat	15-Nov-07	RSDL delayed	2.5	3	0	>24
GD Neat	15-Nov-07	RSDL delayed	3	3	0	>24
GD Neat	15-Nov-07	RSDL delayed	3.5	3	0	>24
GD Neat	8-Nov-07	RSDL delayed	4	2	2	1, 2
GD Neat	15-Nov-07	RSDL delayed	4	3	1	O/N, >24
GD Neat	8-Nov-07	RSDL delayed	6	2	2	1, 1
GD Neat	8-Nov-07	RSDL delayed	8	2	2	1, 1
GD Neat	8-Nov-07	RSDL delayed	10	2	2	1, 1
GD Neat	8-Nov-07	RSDL delayed	12	3	3	1, 1, 1
GD Neat	2-Aug-07	RSDL delayed	15	2	2	1, 1
GD Neat	8-Nov-07	RSDL delayed	15	2	2	1, 1
GD Neat	2-Aug-07	RSDL delayed	30	2	2	1, 1
GD Neat	2-Aug-07	RSDL delayed	60	1	1	1
GD Neat	2-Aug-07	RSDL delayed	60	2	2	1, 1
GD Neat	8-Nov-07	RSDL delayed	60	1	1	1
GD Neat	15-Nov-07	RSDL delayed	60	1	1	1
GD Neat	2-Aug-07	RSDL delayed	90	2	2	1, 1
GD Neat	2-Aug-07	RSDL delayed	120	2	2	1, 1
GD Neat	2-Aug-07	RSDL delayed	150	2	2	1, 1
GD Neat	2-Aug-07	RSDL delayed	180	1	1	2

Table A8. Raw data for delayed decontamination for RSDL animals challenged with 55.0 mg/kg GD (5 LD_{50}s). O/N = overnight (6-20 hrs).